The BioEnergy Science Center: Initial Results in Overcoming Biomass Recalcitrance

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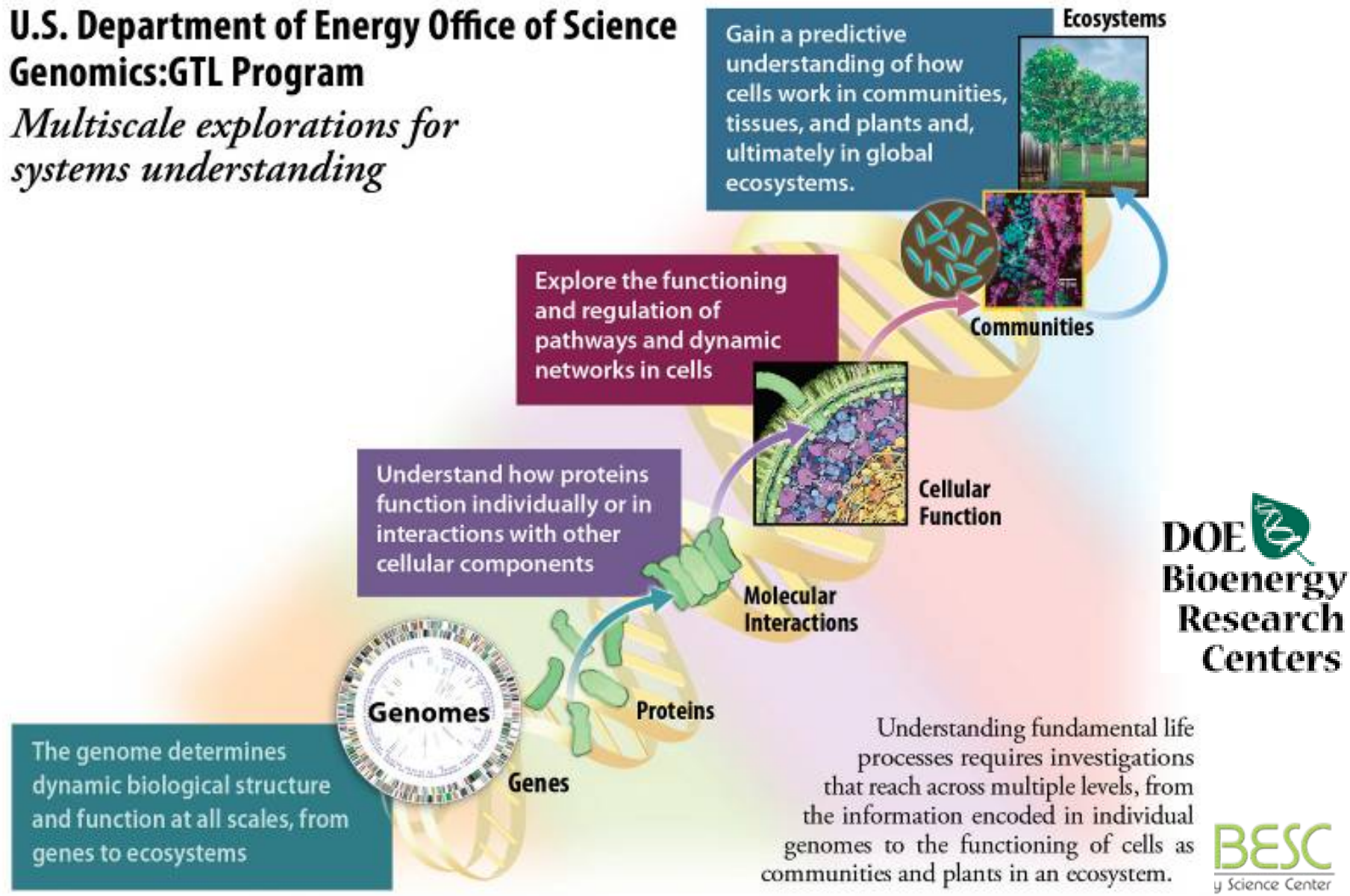
BioEnergy Science Center
http://www.bioenergycenter.org/

Biomass 2009
March 17, 2009

GTL Core Science Goals at All Scales



DOE-GTL Mission Challenges for Biology

U.S. DEPARTMENT OF ENERGY

DOE Bioenergy Research Centers

Biofuels

Gain knowledge and tools for using microbes and plants to build a national biofuel capability to

- Develop sustainable energy crops.
- Develop biotechnologies for producing advanced biofuels

Cleanup

Understand microbial and plant impacts on subsurface contaminant fate to

- Develop better assessment tools.
- Design improved bioremediation methodologies.

Climate Stabilization

Determine ocean and terrestrial ecosystems' contributions to the global carbon cycle to

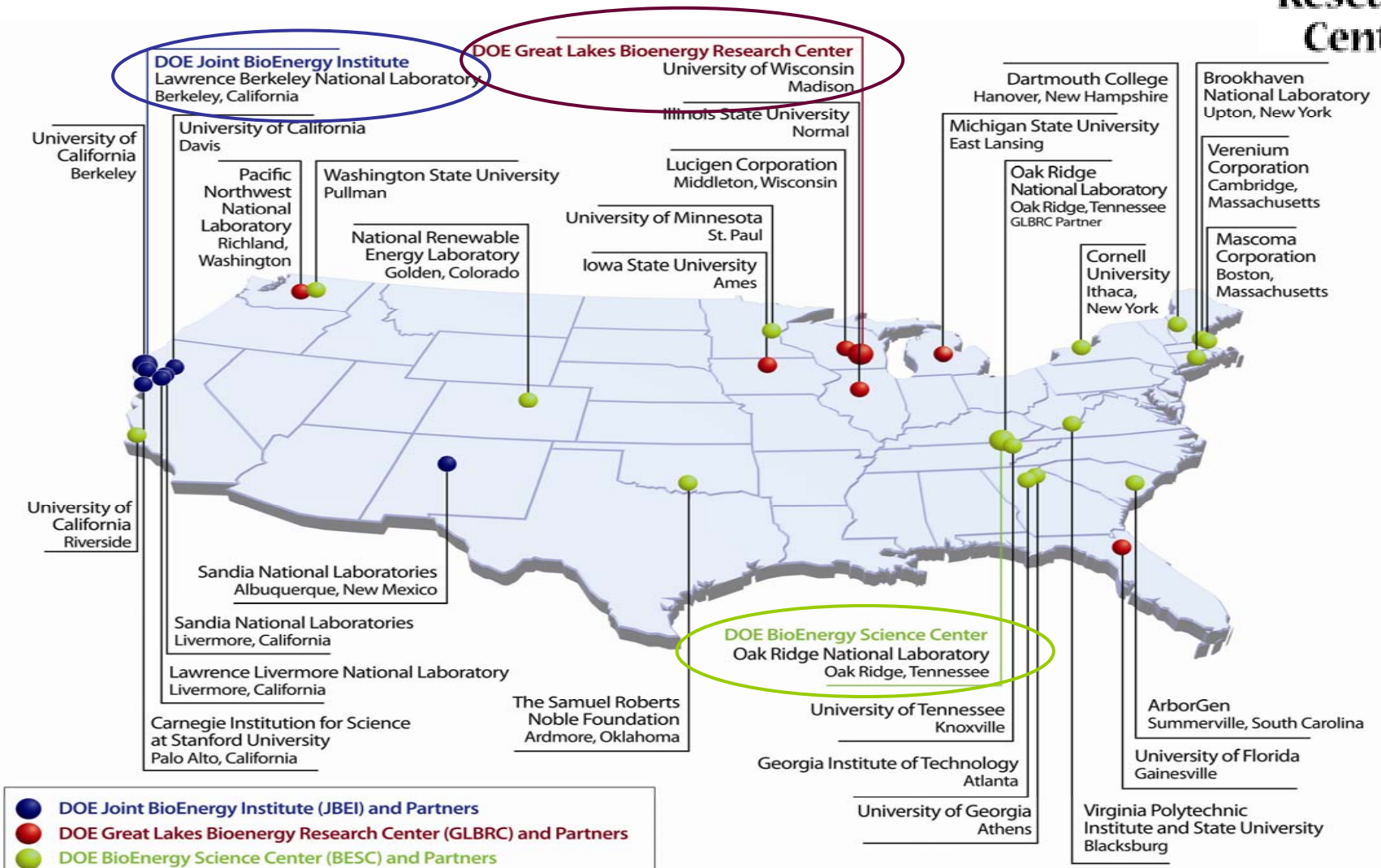
- Improve projections of climate change and its impacts.
- Create carbon-biosequestration strategies.

BESC BioEnergy Science Center

DOE Bioenergy Research Centers: Multi-Institutional Partnerships

U.S. DEPARTMENT OF ENERGY

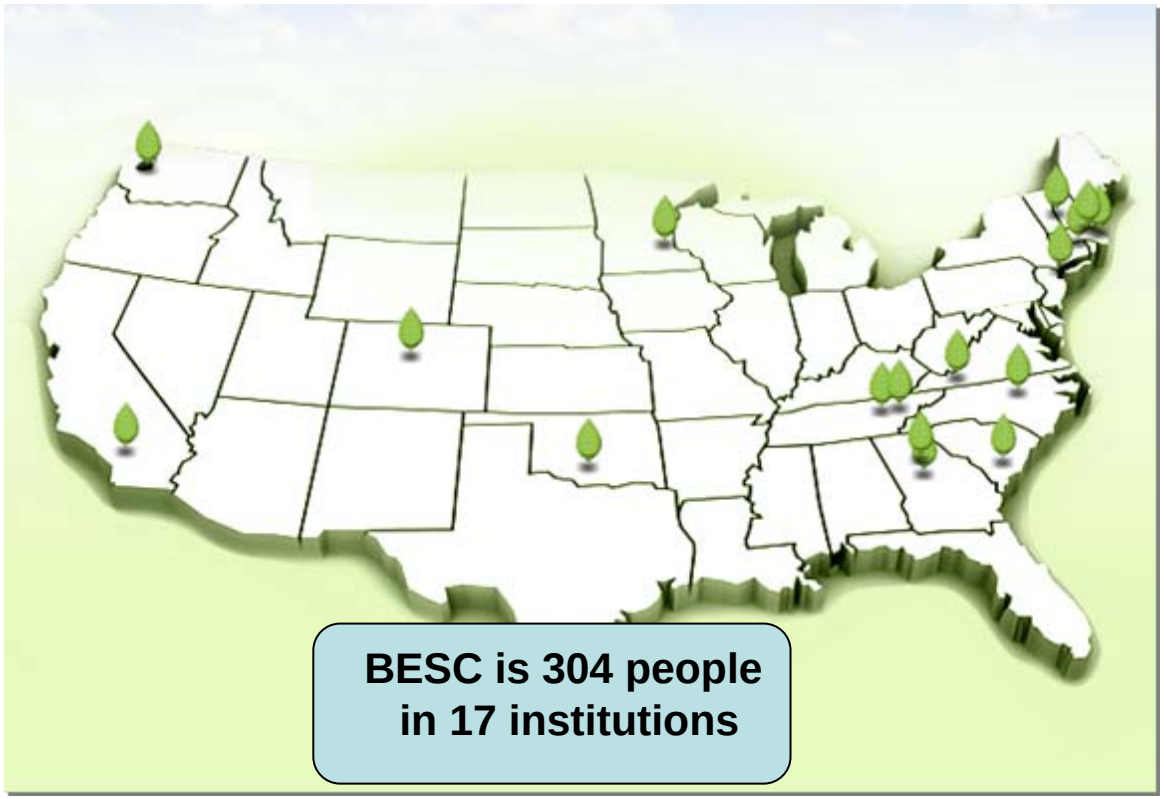
DOE Bioenergy Research Centers



The BioEnergy Science Center

U.S. DEPARTMENT OF ENERGY

BESC: A multi-institutional DOE-funded center dedicated to <u>understanding and modifying plant biomass recalcitrance</u>

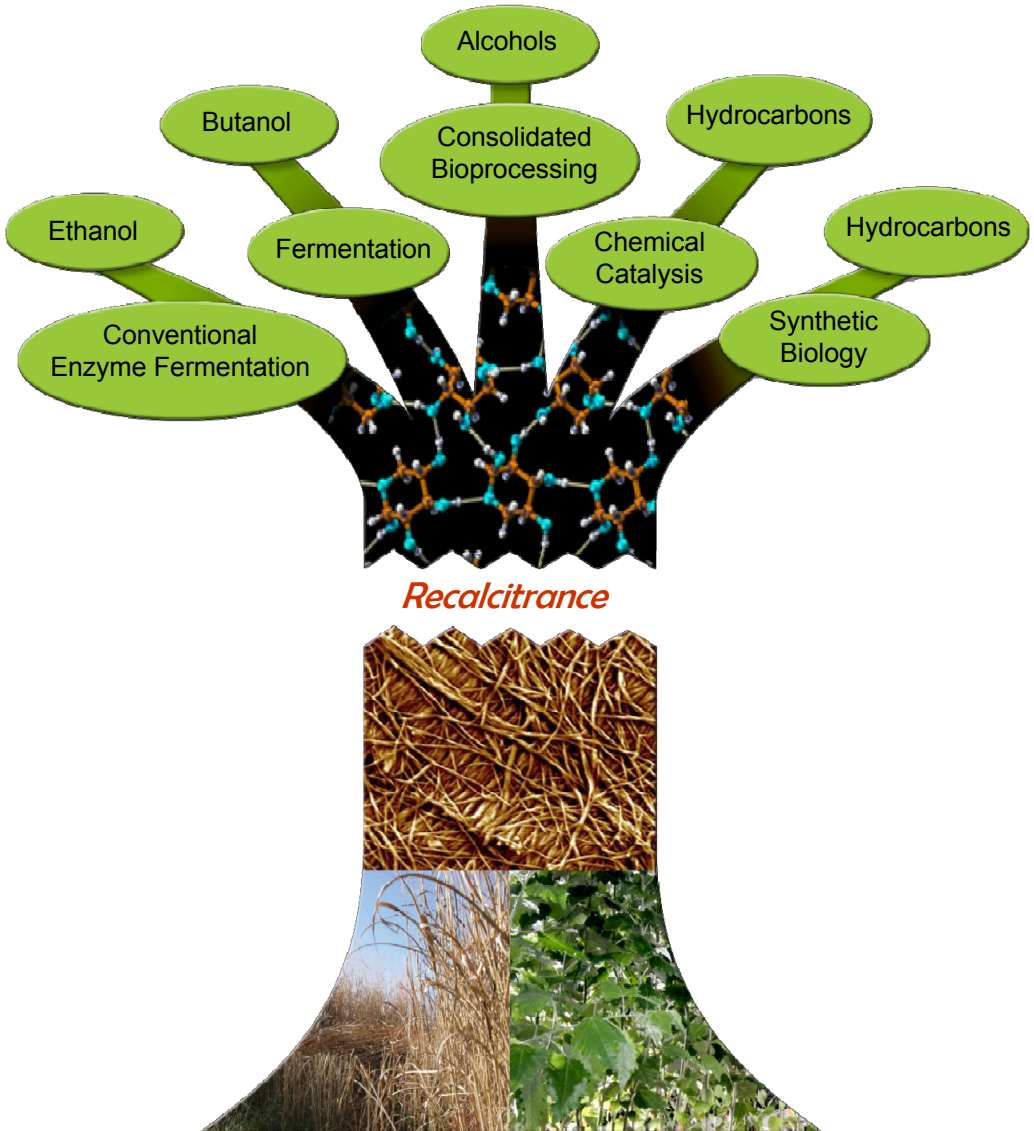


- Oak Ridge National Laboratory
- University of Georgia
- University of Tennessee
- National Renewable Energy Laboratory
- Georgia Institute of Technology
- Samuel Roberts Noble Foundation
- Dartmouth College
- ArborGen, LLC
- Verenium Corporation
- Mascoma Corporation
- University of California-Riverside
- Cornell University
- Washington State University
- University of Minnesota
- North Carolina State University
- Brookhaven National Laboratory
- Virginia Polytechnic Institute

http://www.bioenergycenter.org/

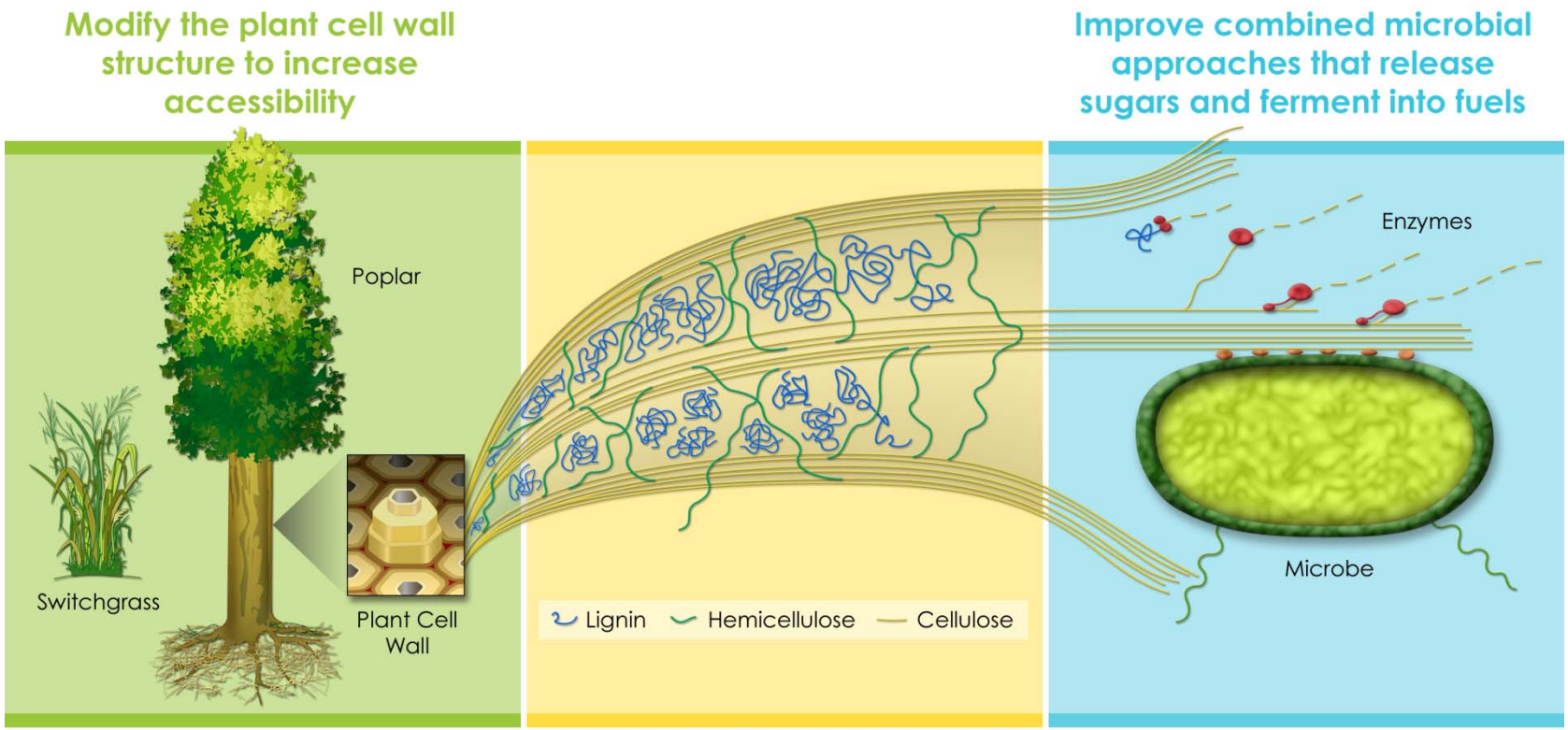
BESC BioEnergy Science Center

Access to the Sugars in Lignocellulosic Biomass is the Current Critical Barrier



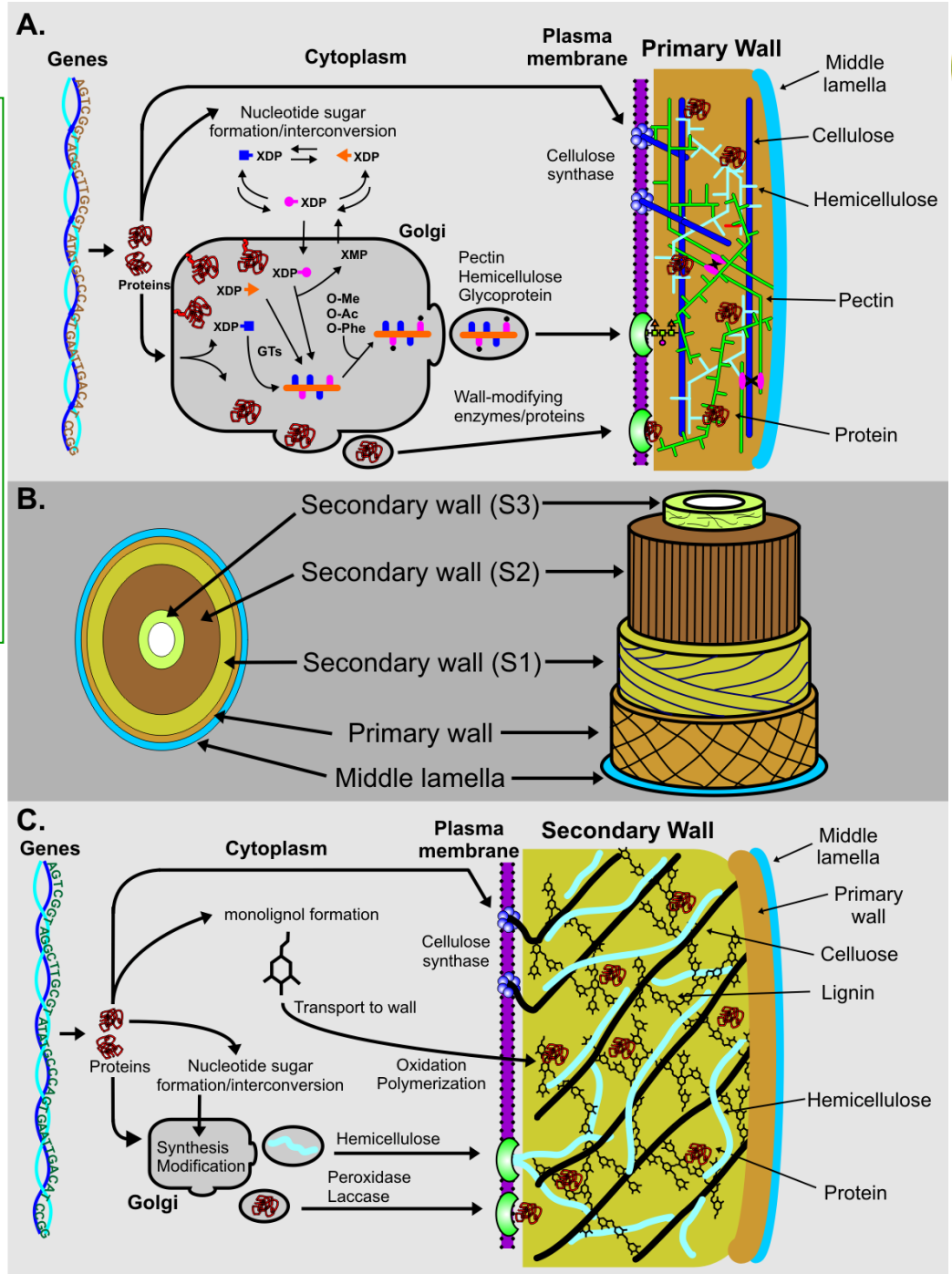
- Solving this will cut processing costs significantly and be used in most conversion processes
- This requires an integrated multidisciplinary approach
- Timeframe
  - Modified plants to field trials: Year 5
  - New or improved microbes to development: Years 4−5
  - Analysis and screening technologies: Year 3 on

A Two-pronged Approach to Increase the Accessibility of Biomass Sugars



Both utilize rapid screening for relevant traits followed by detailed analysis of selected samples

Functional modifications of both primary and secondary cell walls may decrease recalcitrance



Malcolm O'Neill, CCRC
Mohnen et al. (2008)

U.S. DEPARTMENT OF ENERGY

Primary Wall

90% polysaccharide

Dividing and growing cells

Pectin
Hemicellulose
Cellulose
(proteins)

Secondary Walls

70 – 80% polysaccharide

Some cells with structural roles

↓ Pectin
Hemicellulose
Cellulose
Lignin
(proteins)

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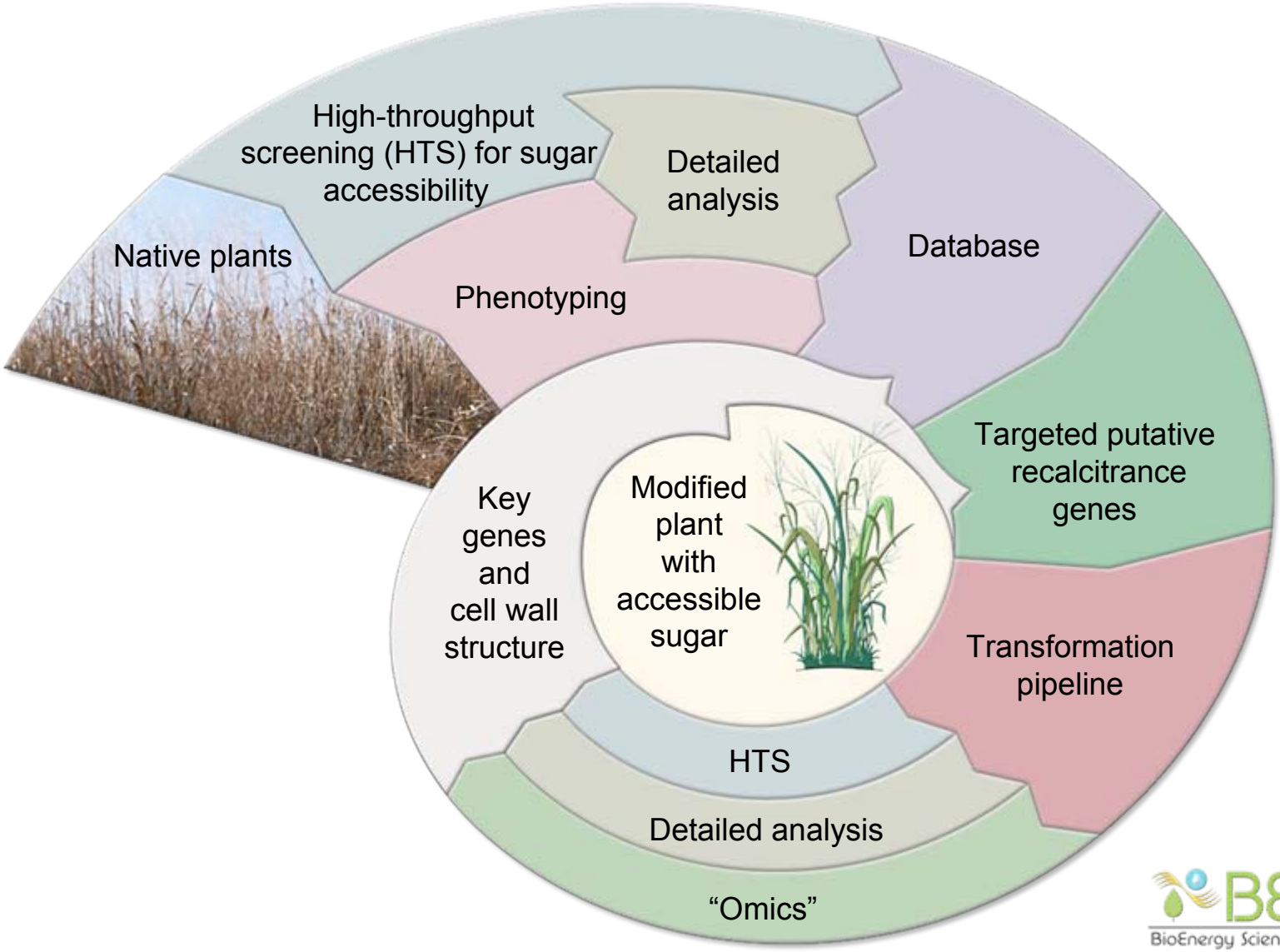
What Genes Control Cell Wall Synthesis (and Access to the Sugars)?

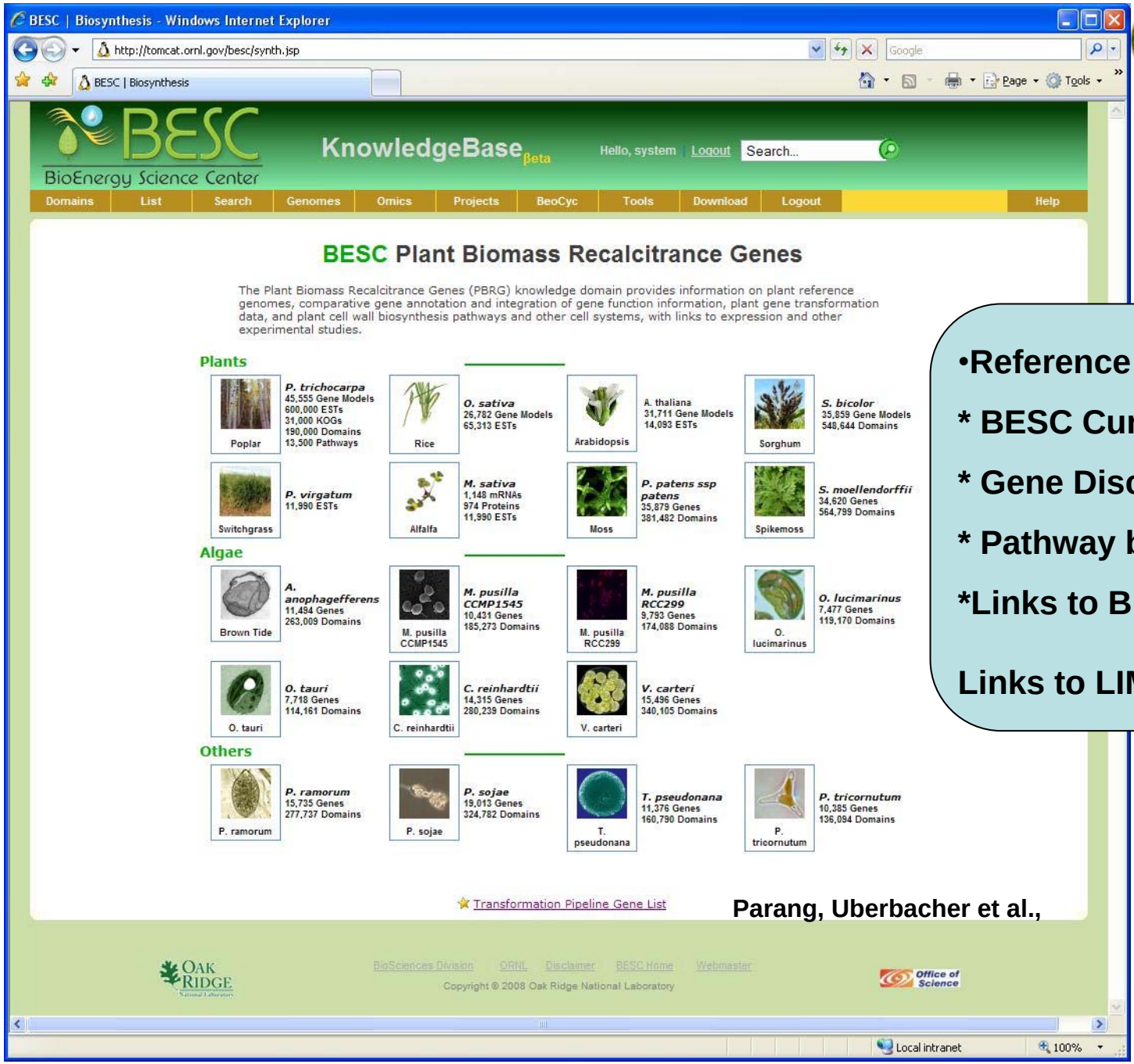


Targeted Plant Genes and Transformation Pipeline

- Gene transformation pipeline established and running
  - 70 *Populus* genes per set
  - 4 Switchgrass for stable transformation per set
  - 30 Switchgrass by VIGS (viral induced gene silencing) per set
  - Three sets totaling >300 genes in pipeline after three rounds of review
- *Populus*
  - Transformation: 200 genes per year
  - Activation Tagging: 1000 genes per year
- Switchgrass
  - Transformation: 20 genes Year 1; 40-60 Year 2
  - VIGS: 200 genes per year, RNAi
- Higher perennial plants have fewer genetic tools and so targets must be selected carefully

Functions of initial targets (set #1)

Functional category	# genes
Cell wall biosynthesis	50
Cell division and expansion	46
Signal transduction	26
Stress response	20
Metabolism	19
Intracellular traffic	9
Protein fate	9
Transcription	9
Plant defence	4
Nucleic acid or nucleotide binding	2
Transporters	2
Total	196



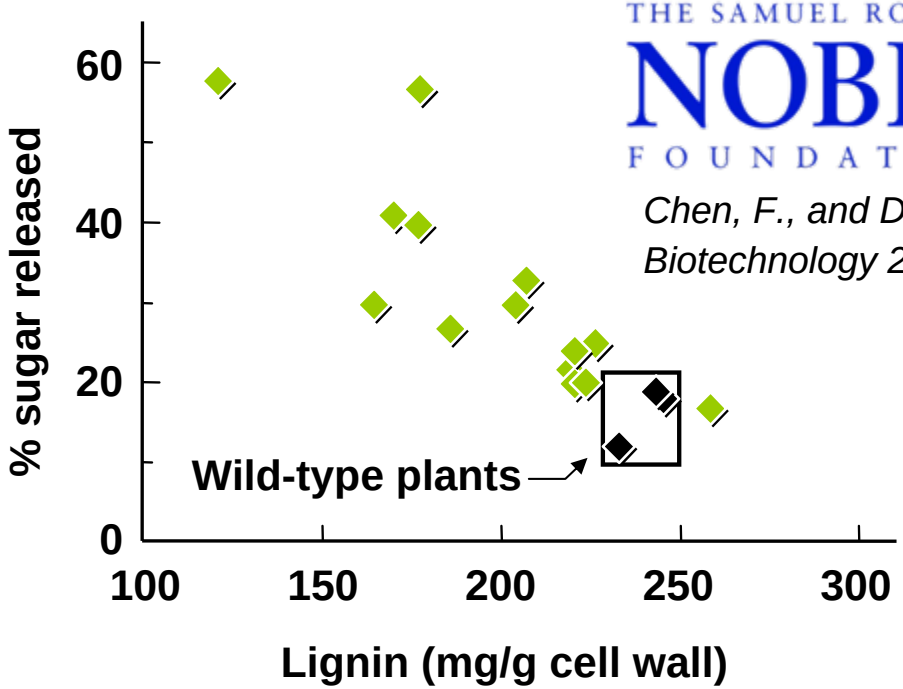
- Reference Genomes
- * BESC Curation
- * Gene Discovery Tools
- * Pathway building
- *Links to BESC data

Links to LIMS system

Parang, Uberbacher et al.,

Targeted Cell Wall Synthesis Approach: A Few Examples

Modifying Cell Wall Composition and Structure Can Reduce Recalcitrance



THE SAMUEL ROBERTS NOBLE FOUNDATION

Chen, F., and Dixon, R.A. (2007) Nature Biotechnology 25, 759-761.

- More sugar is solubilized by cellulase when the lignin content of alfalfa cell walls is reduced
- Strategy is feasible for *Populus* and switchgrass

Discovery-based Approach to Identify Recalcitrance-Associated Genes via Analysis of Natural Variation

Mining Genetic Variation in Switchgrass

U.S. DEPARTMENT OF ENERGY

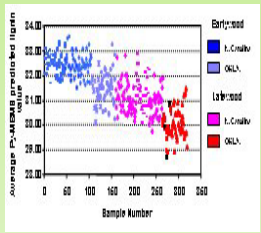
Create diverse population by cross "lowland" SG AP-13 and "upland" SG VS-16 into 385 pseudo F1 clones

Pseudo F_1 population of 385 genotypes

Clones ready for field planting

HTS Pipeline

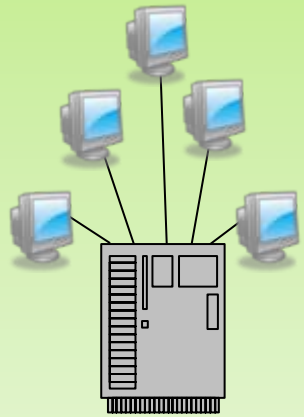
Sugar Release Assay



Analytical Pyrolysis

Create Genetic Marker Map to identify allelic variation

Identify Marker Trait Association



Cell Wall Biosynthesis Database

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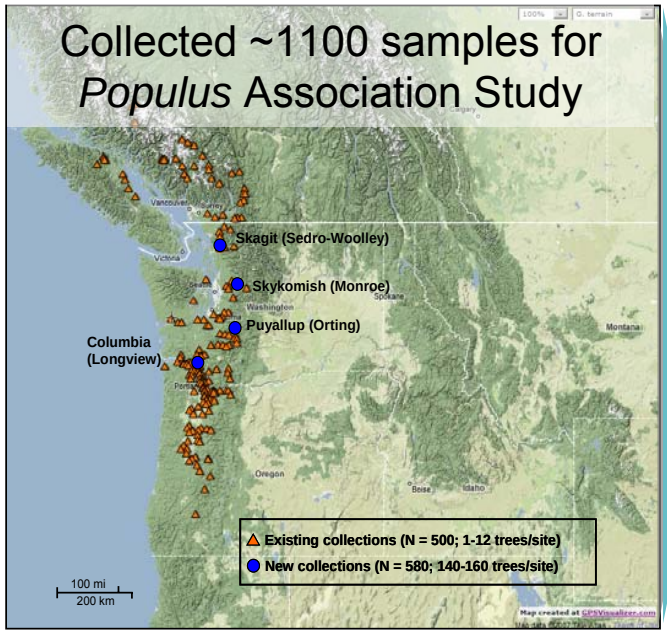
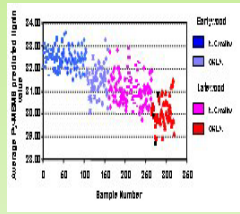
Mining Variation to Identify Key Genes in Biomass Composition and Sugar Release



HTS Pipeline

Sugar Release Assay



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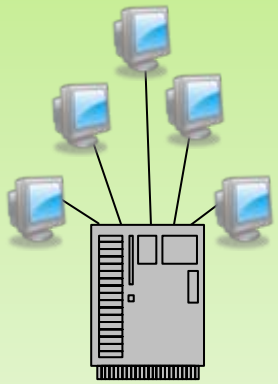


Cell Wall Biosynthesis Database

Establish common gardens for association and activation tag populations with 1000s of plants

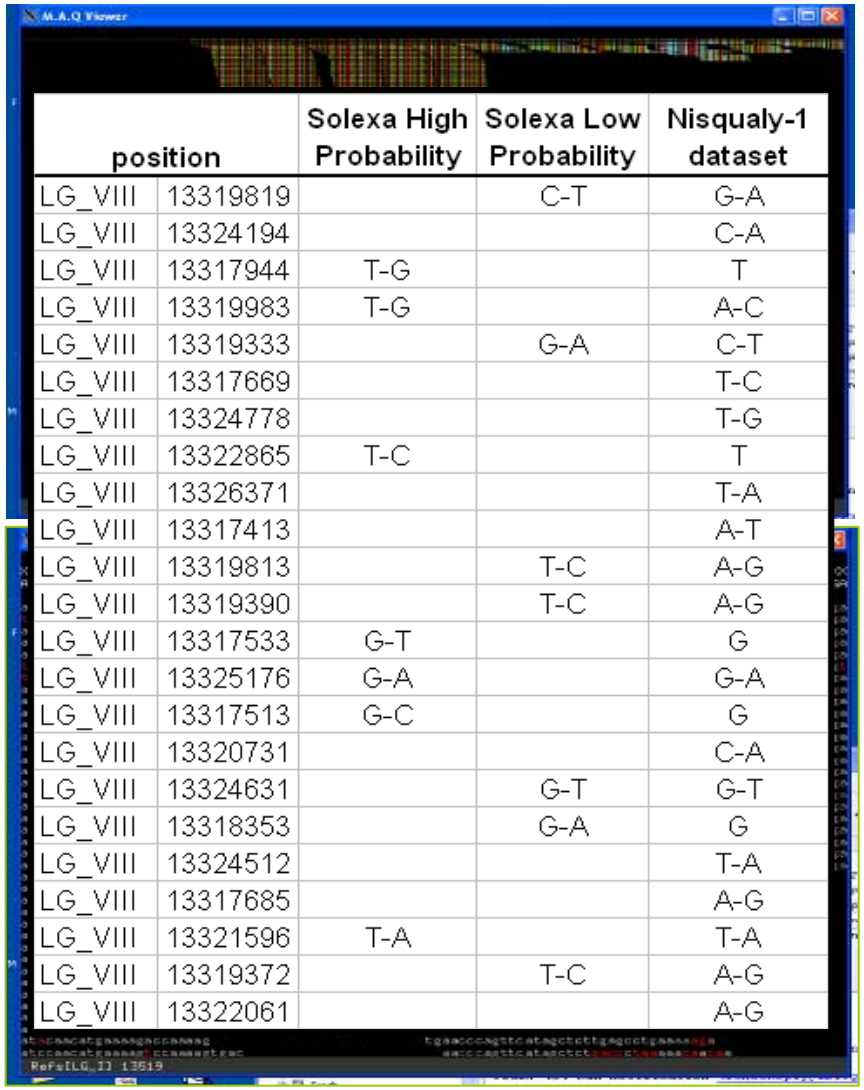
OAK RIDGE National Laboratory

BESC BioEnergy Science Center

Association Genetics Study –

Whole-genome Resequencing in effort to discover SNPs across *Populus* genome

In collaboration with the Joint Genome Institute 10 alternate *Populus* genomes are being resequencing



position		Solexa High Probability	Solexa Low Probability	Nisqualy-1 dataset
LG_VIII	13319819		C-T	G-A
LG_VIII	13324194			C-A
LG_VIII	13317944	T-G		T
LG_VIII	13319983	T-G		A-C
LG_VIII	13319333		G-A	C-T
LG_VIII	13317669			T-C
LG_VIII	13324778			T-G
LG_VIII	13322865	T-C		T
LG_VIII	13326371			T-A
LG_VIII	13317413			A-T
LG_VIII	13319813		T-C	A-G
LG_VIII	13319390		T-C	A-G
LG_VIII	13317533	G-T		G
LG_VIII	13325176	G-A		G-A
LG_VIII	13317513	G-C		G
LG_VIII	13320731			C-A
LG_VIII	13324631		G-T	G-T
LG_VIII	13318353		G-A	G
LG_VIII	13324512			T-A
LG_VIII	13317685			A-G
LG_VIII	13321596	T-A		T-A
LG_VIII	13319372		T-C	A-G
LG_VIII	13322061			A-G

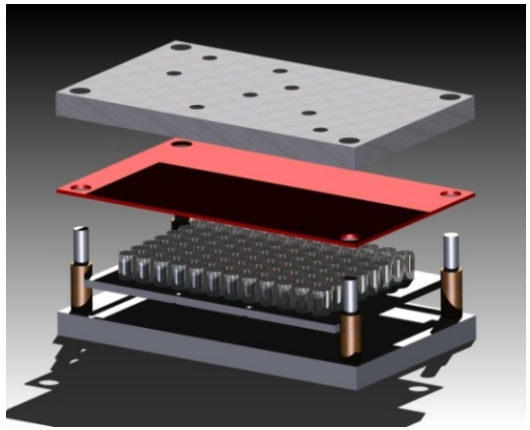
Preliminary Results

- **28x depth from 6 Solexa runs**
- **85% align to Nisqually-1**
- **843,000 SNP loci relative to reference**
- **78,000 SNP loci are heterozygous**

HTP Characterization Pipeline for the Recalcitrance Phenotype

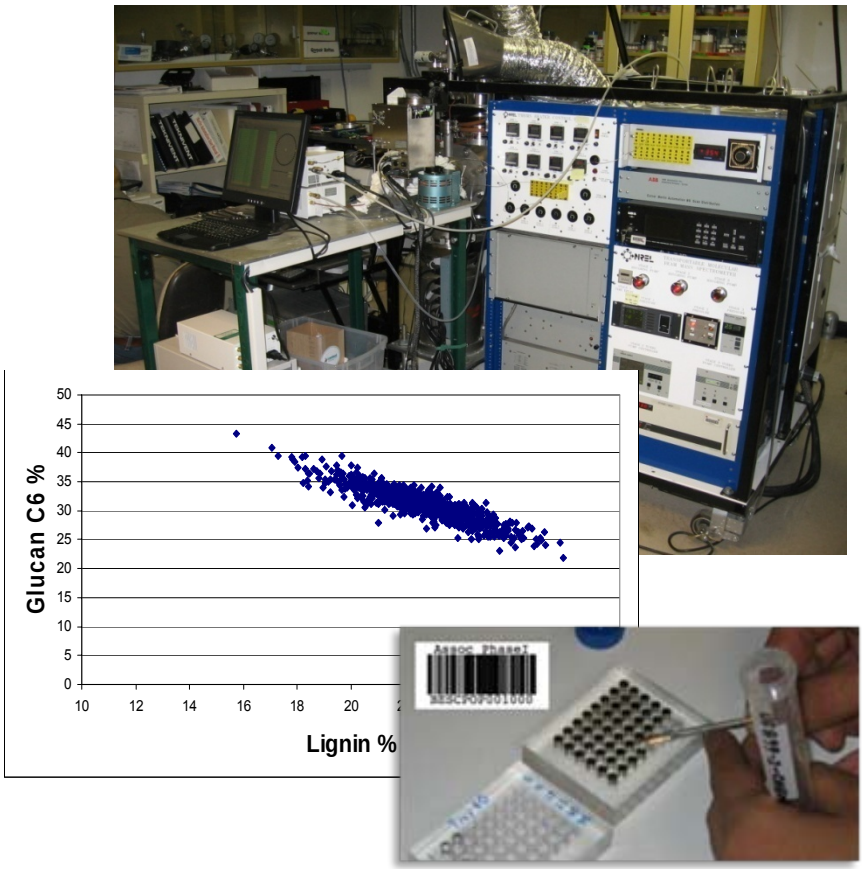
- Screening of 1000's of samples

Composition analytical pyrolysis, IR, confirmed by wet chemistry → Pre-treatment new method with dilute acid and steam → Enzyme digestibility sugar release with enzyme cocktail



Detailed chemical and structural analyses of specific samples

Composition Data from Analytic Pyrolysis (MBMS) for High-throughput Screening of Transgenic Populations

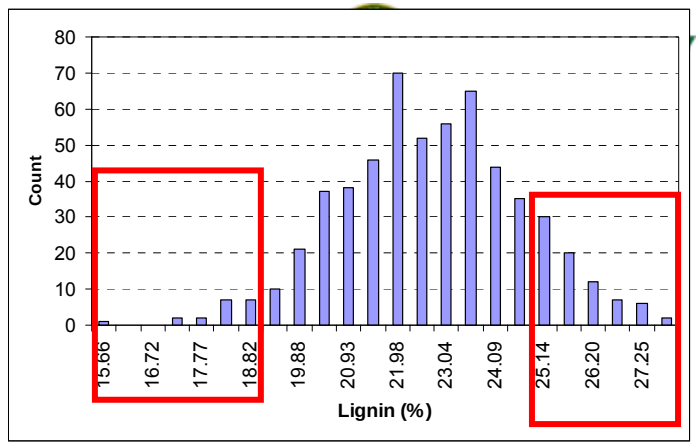


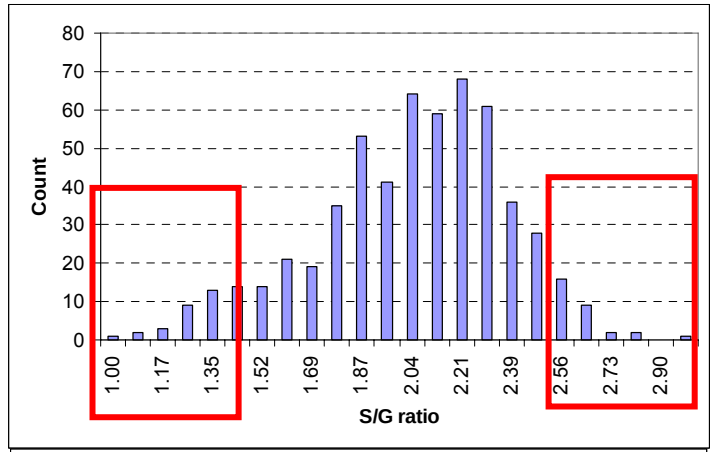
Composition data from *Populus* association study (798 samples) represents full range of known *Populus* variation

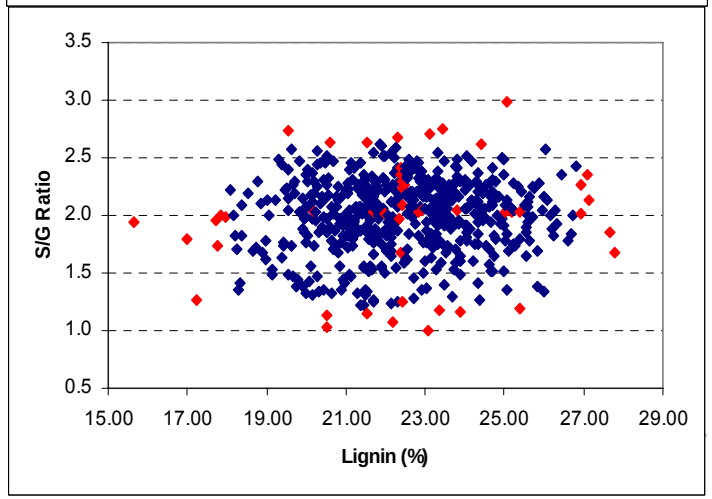
- Rapid (50/h w/ 4mg)
- Reliable
- Gives values for glucan, xylan, lignin, and details on monomers – e.g., S/G
- Complements time-consuming and more variable wet chemistry, molecular and biochemical analyses

Composition Data from *Populus* Association Study

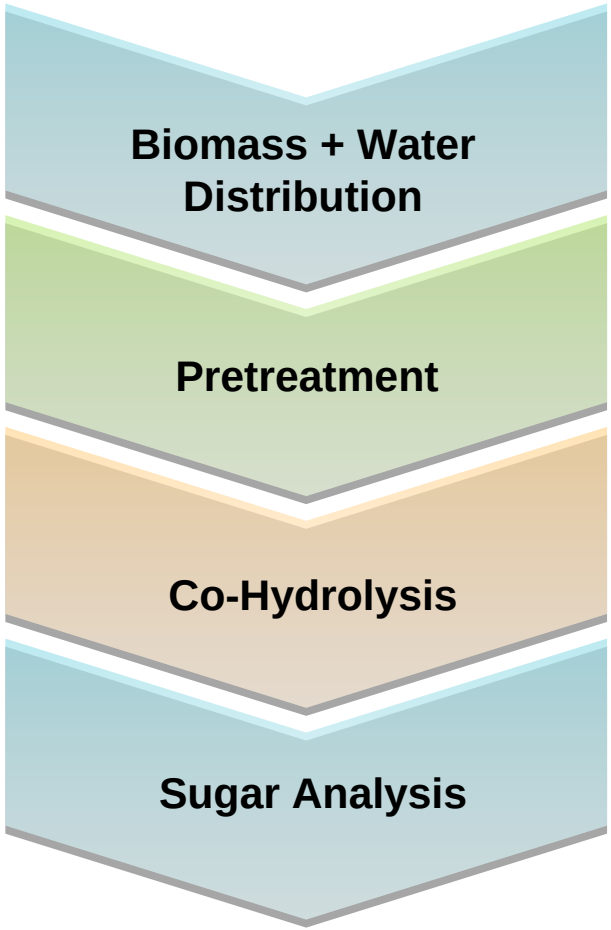
- The association samples display extreme variation in lignin, S/G ratio, and sugar content
- There is a negative correlation between sugar content and lignin content
- All sampled genotypes are being replicated and will be established in a common garden experiment







Enabling Technology: An HTP Pretreatment for 1000s of Small Samples

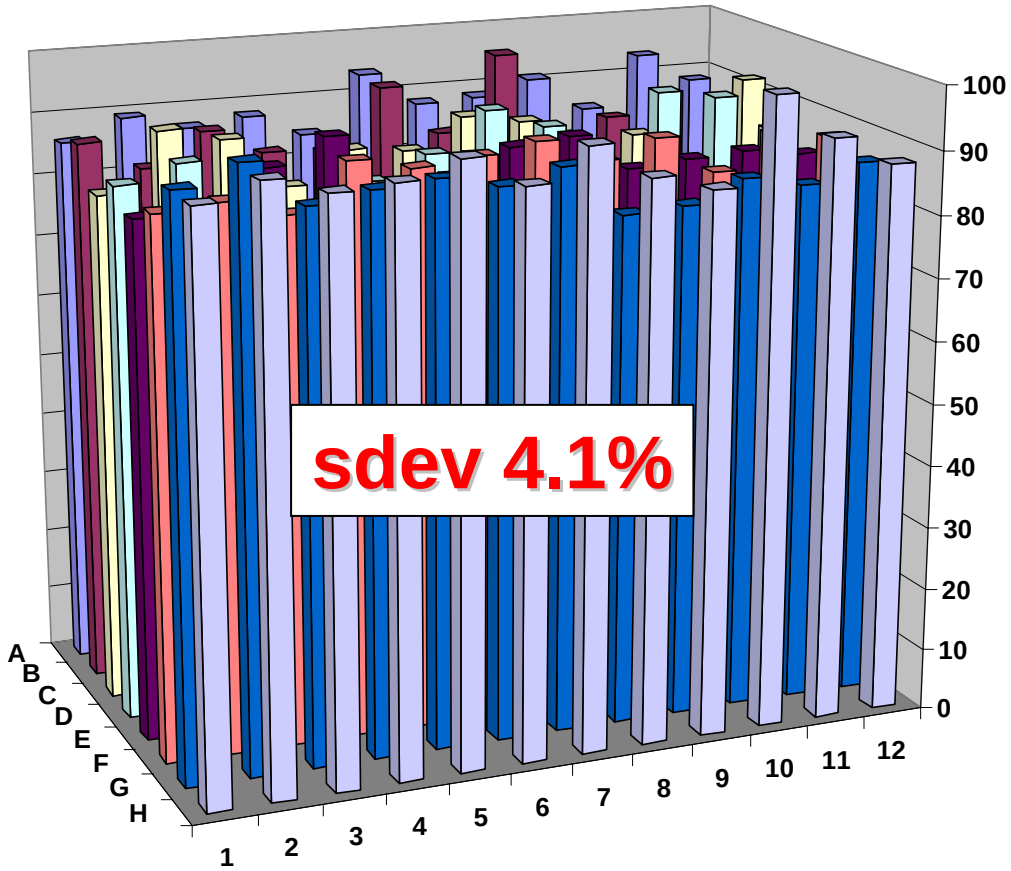


Studer *et al*., presented at the 30th *Symposium on Biotechnology for Fuels and Chemicals*, May 2008

Unique and Important

- *Steam:* efficient uniform heating
- *No separation*: saves time and increases accuracy
- *2-4mg sample size:* reduces material costs

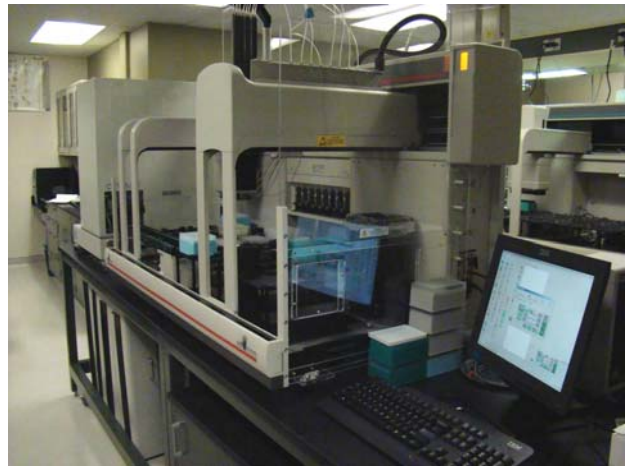
Proof of Concept: 96 well-plate Pretreatment and Co-hydrolysis Performance

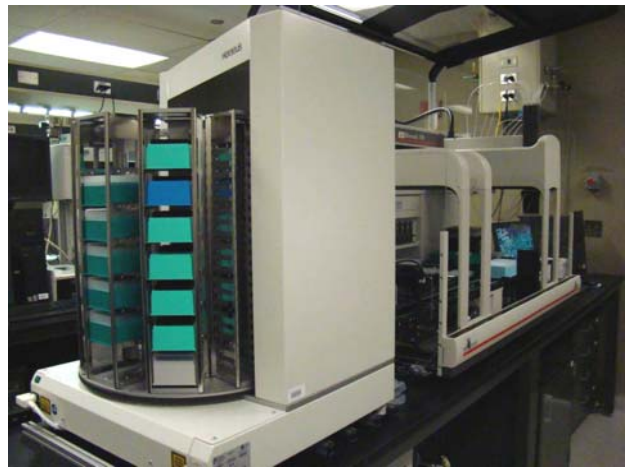


- Poplar (NRELHPT00001)
- Water-only pretreatment at 180°C for 55Min
- 1%w/w solids loading
- Co-hydrolysis: 75 + 25mg of cellulase and xylanase / g of glucan + xylan in the raw biomass
- Heated with steam

HTP Enzymatic Digestion Assays

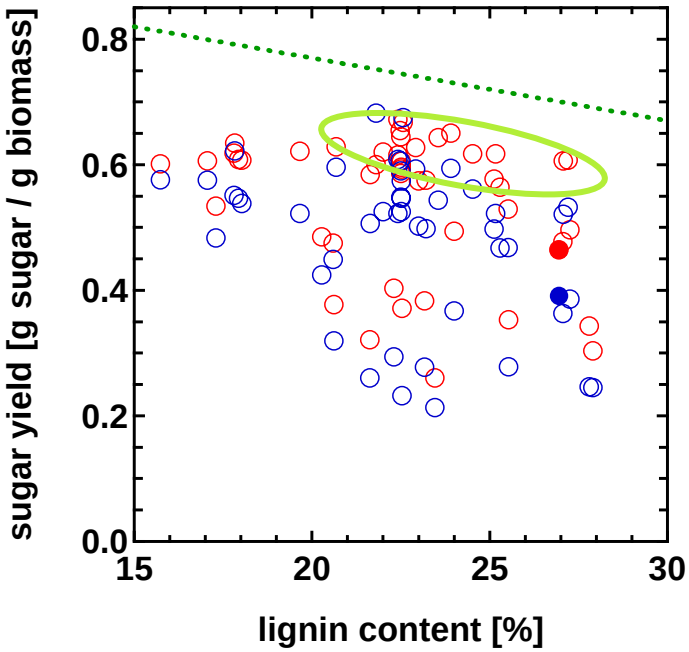
- Recalcitrance is ultimately determined by enzyme access to carbohydrates and sugar release
- HTP assays are needed to assess recalcitrant phenotypes and to screen for more effective enzymes
- 1st tier assays:
  >1000 samples/week
  - Evaluate base-line susceptibility of pretreated biomass as well as enzymes from natural diversity
- 2nd tier assays: <200 samples/day

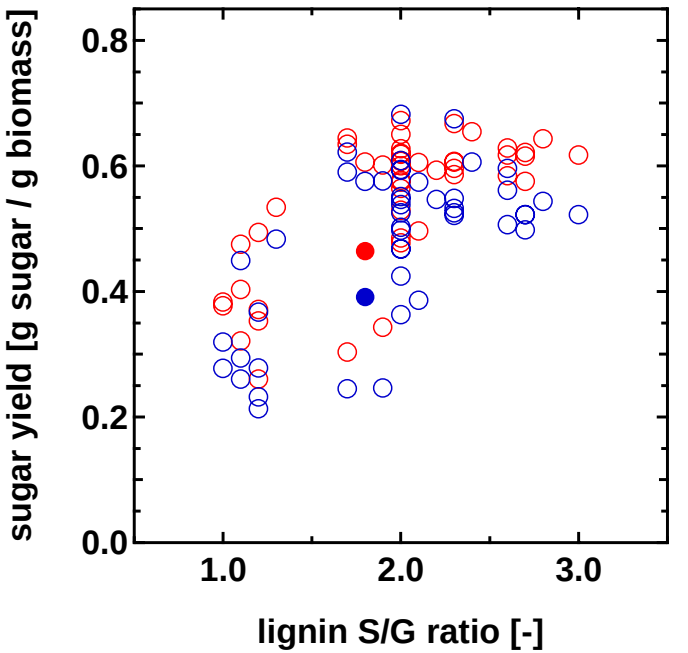




Response surface output for multi-dimensional digest

Populus Association Study





- **Tested for enhanced sugar release characteristics through pretreatment and enzymatic hydrolysis**
  - Hot water pretreatments at **160** and **180°C**
- **HTP pretreatment and co-hydrolysis in 96 well-plates**

- **Preliminary observations:**
  - **Sugar yield increases with S/G ratio**
  - **Lignin content has minimal effect**
  - **Some outlier poplar samples exhibit very high sugar release**
- **Characterization pipeline works**

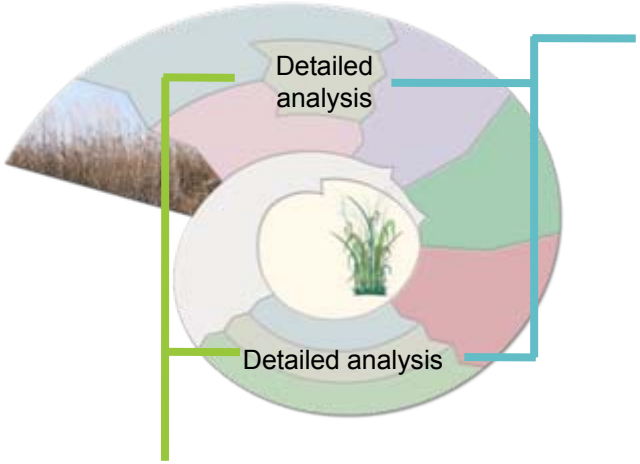
Pretreatment conditions: ○ **180°C, 18Min** ○ **160°C, 68Min**

●● **Standard BESC poplar**

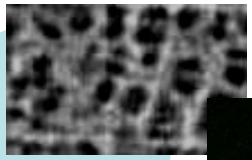
···· **Theoretical sugar yield**

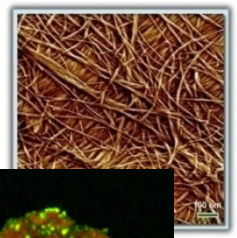
***Studer*, Wyman et al.**

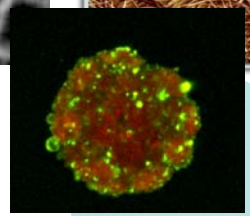
Detailed Analysis of Specific Samples Inform Cell-wall Chemistry and Structure



Imaging





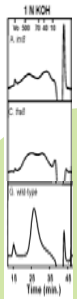


AFM of switchgrass showing cellulose microfibrils

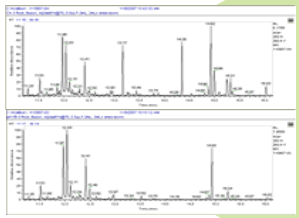
Bio-ultraCAT for 3-D density of *Populus* cell walls

Immunolocalization using wall antibodies on *Populus* protoplasts

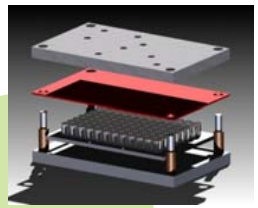
Chemistry



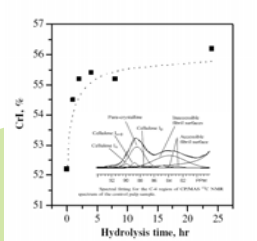
Fractionation and chromatography



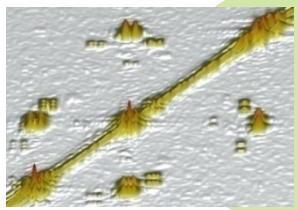
Mass Spectrometry for key metabolites



2nd tier Enzyme and Pretreatment tests



NMR for cellulose crystallinity



2D ^{1}H-NMR sees altered bonds in polysaccharides and lignin in biomass

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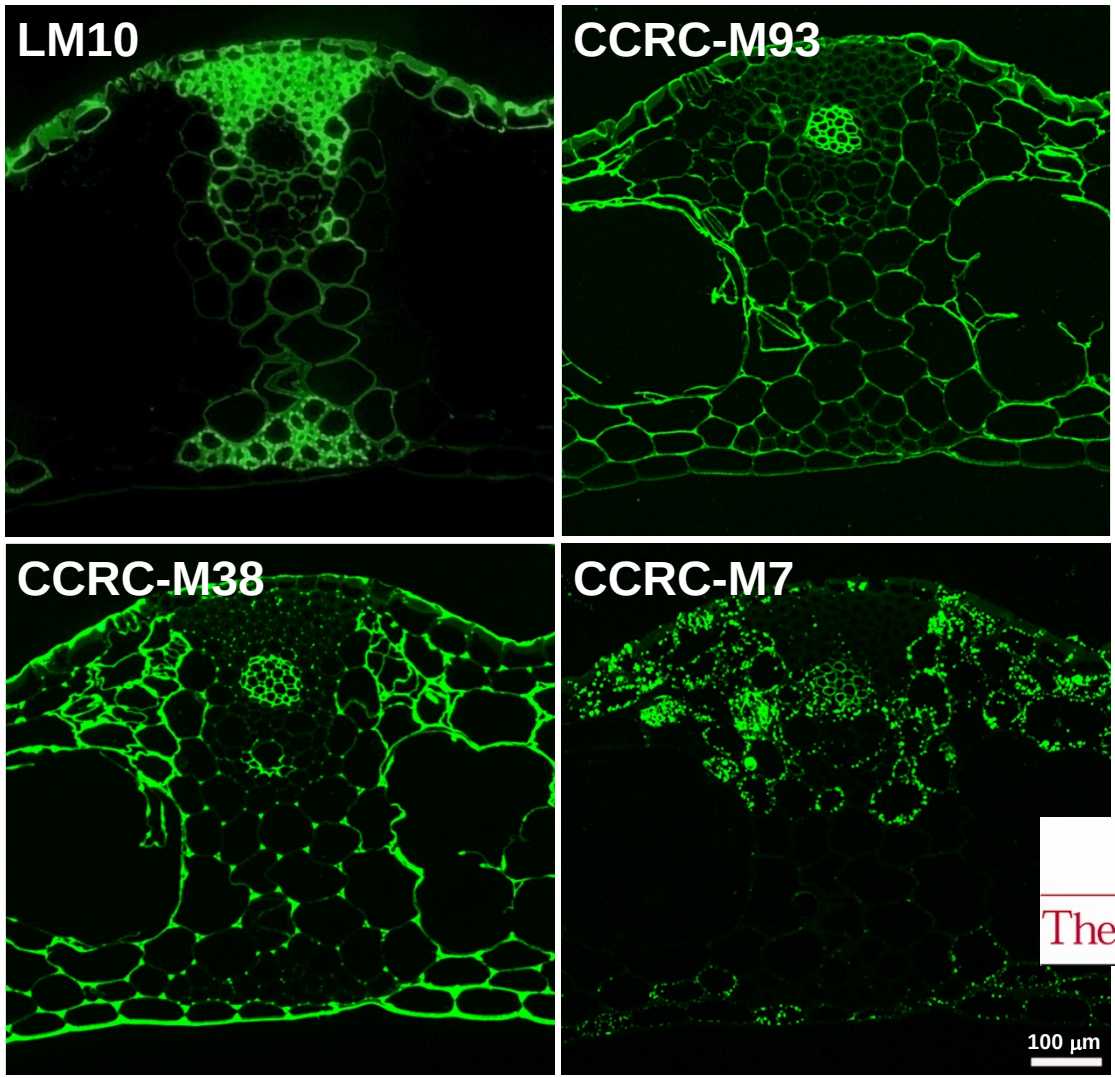
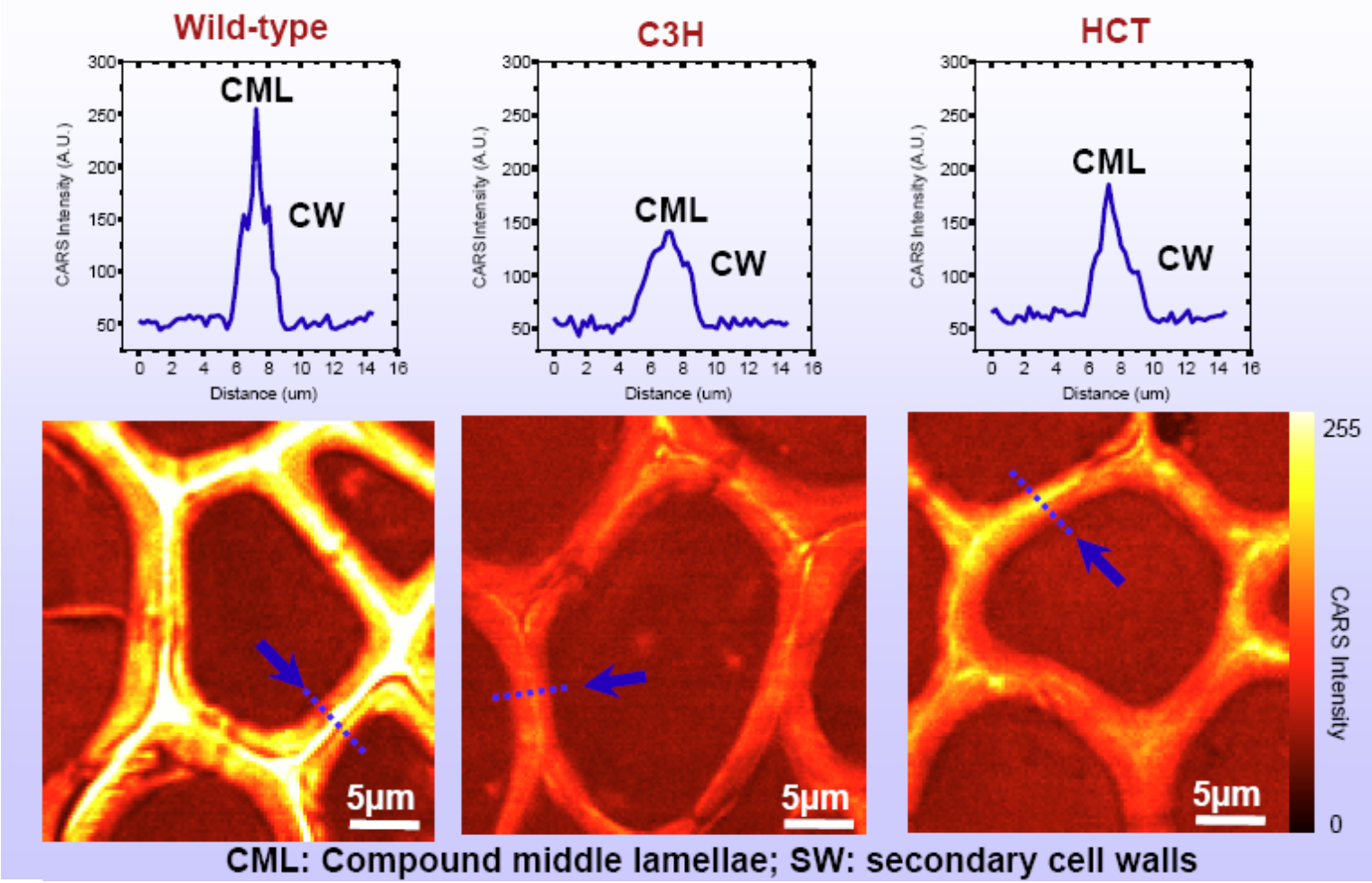
Immunolocalization of Different Polysaccharide Structures in Leaves of Switchgrass Using Wall-epitope Specifc Monoclonal Antibodies

Utku Avci, Michael Hahn

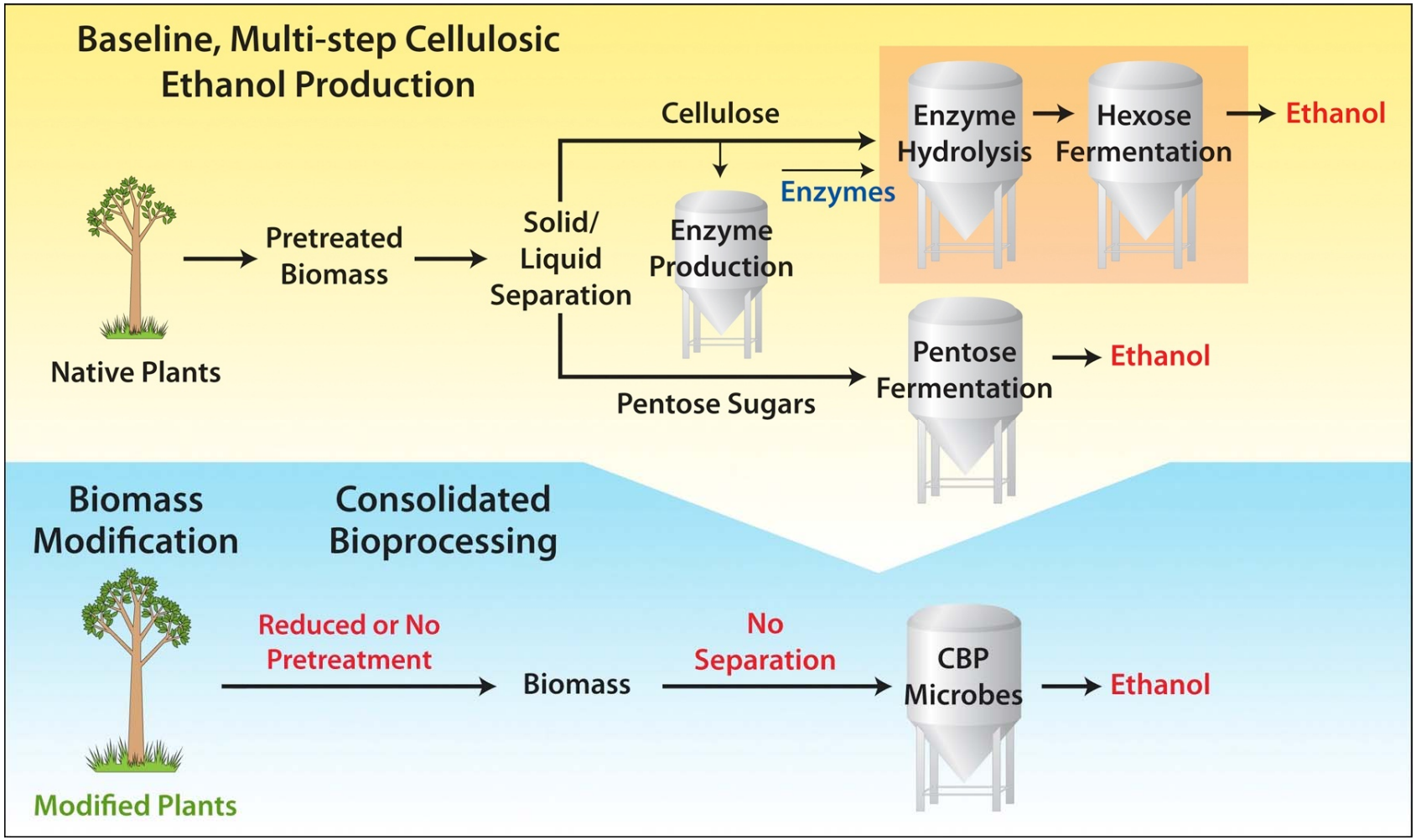


CARS (Coherent Anti-Stokes Raman Scattering) Imaging of Lignin in Interfascicular Fiber Cell Walls in Alfalfa

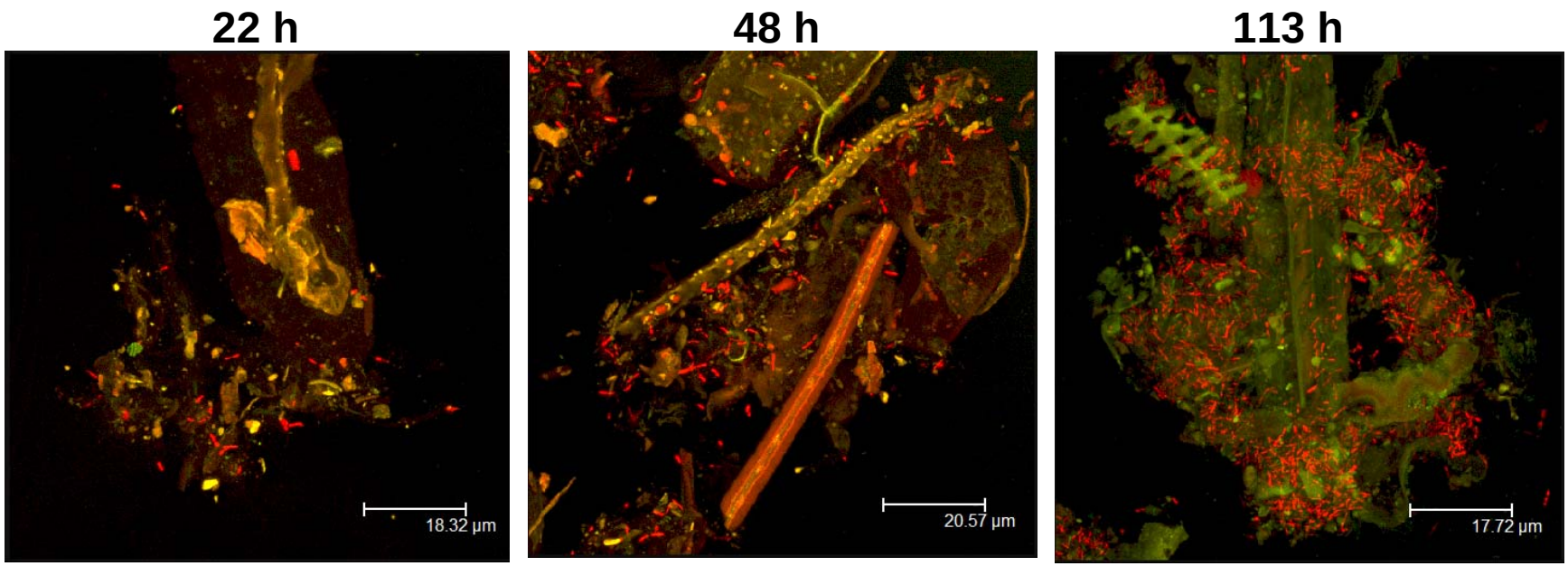


S-Y Ding (NREL) and X. S. Xie (Harvard)
tools under BER imaging grant; sample analysis under BESC, MS in preparation

BESC Will Revolutionize How Biomass is Processed



Temporal Dynamics of *Clostridia thermocellum* Attachment to Pretreated Switchgrass



Cells were collected at various timepoints during fermentation, frozen*, and then stained with Syto9 and Propidium iodide. Z-series optical sections were collected using confocal microscopy and projected as a single 2D image. Overlays of the green (Syto9) and red (PI) channels are shown for each timepoint. Cells provided by B. Raman. *Since cells were frozen, most stain with PI (dead)

Thank you
Retreat December 2008

DOE Bioenergy Research Centers

DOE GENOMICS:GTL
SYSTEMS BIOLOGY FOR ENERGY AND ENVIRONMENT
OFFICE OF SCIENCE U.S. DEPARTMENT OF ENERGY

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BESC is a U.S. Department of Energy Bioenergy Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science

BESC
BioEnergy Science Center

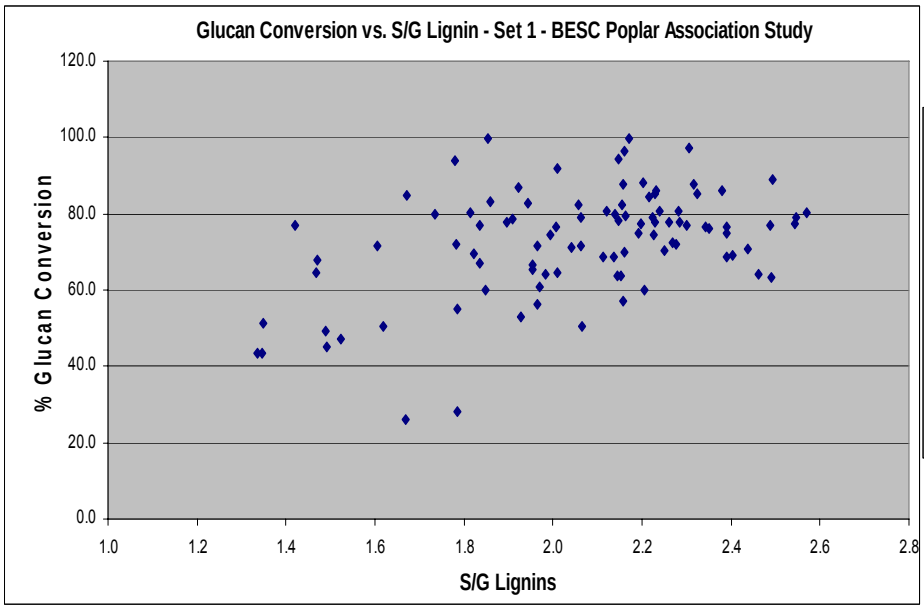
Highlights 2008-till February 2009

- ***138+*** Scientific presentations at meetings and conferences worldwide
- **29** Scientific publications
- **11** Workshops and seminars for BESC researchers and graduate students
- **10** Inventions disclosed which are under evaluation by the BESC Commercialization Council and 2 additional in-preparation
- Scientific collaboration with the University of British Columbia has contributed over 250 additional *Populus* samples at no cost to BESC
- ***70+*** Presentations to Stakeholders (Under Secretaries, Congressmen and Staff Members, Businessmen, etc.)
- ***65+*** Television, Print, and Radio Interviews
- Education program with the Creative Discovery Museum in Chattanooga, Tennessee to develop a Biofuels Outreach Lesson
- Co-sponsored Global Venture Challenge 2008 in April at ORNL

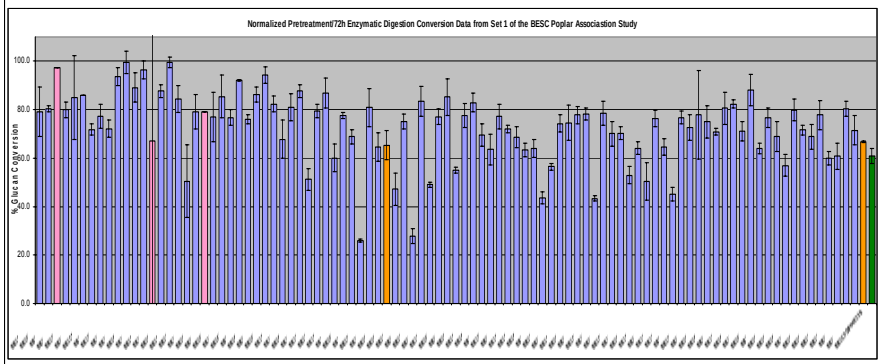
HTP Pretreatment/Saccharification Analysis of Poplar Diversity

- **Notes on General Set-Up**
  - 95 different samples run in triplicate + stds and controls
  - Biomass load at 5±0.3 mg per reactor well
  - Pretreatment run at 180°C for 40 min
  - Digestion w 100mg Spezyme/g biomass for 72 h
  - Glucose analysis by glucose oxidase assay
  - Data normalized to conversion rates for the BESC poplar std

T-test probability that groups show trend is >99.5%



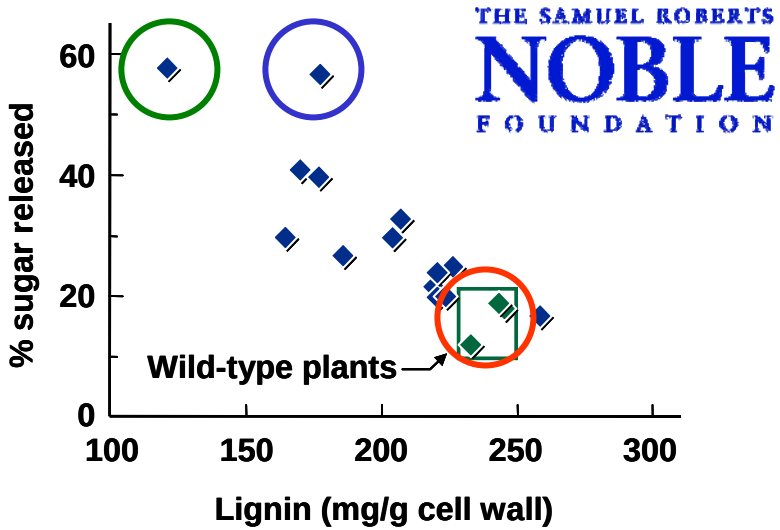
Raw data

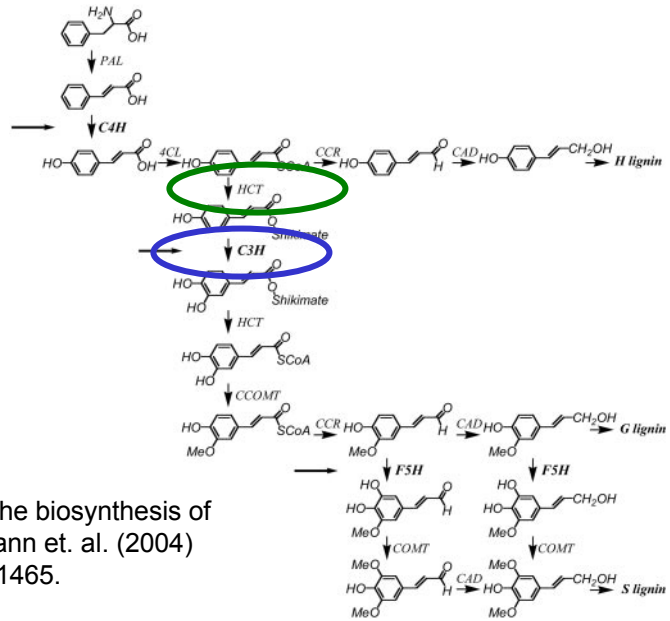


Mike Himmel, Steve Decker, Mark Davis et al.

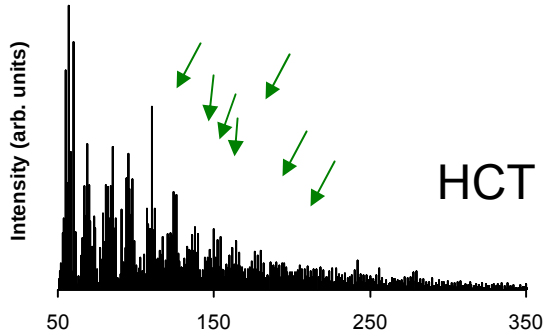
Analytical Pyrolysis of Low Lignin Alfalfa

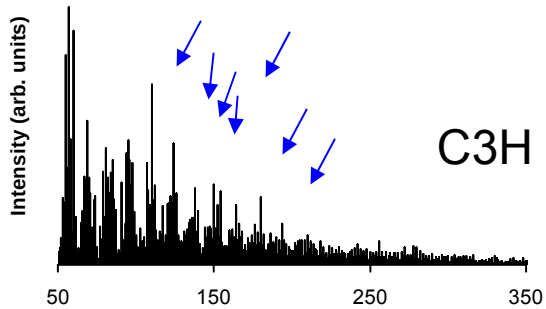
36 minutes of analysis for 6 (x3) samples

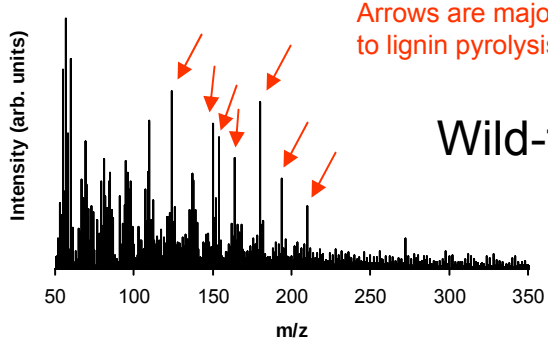




Current scheme for the biosynthesis of monolignols. (Hoffmann et. al. (2004) Plant Cell 16, 1446–1465.







Arrows are major peaks assigned to lignin pyrolysis fragments

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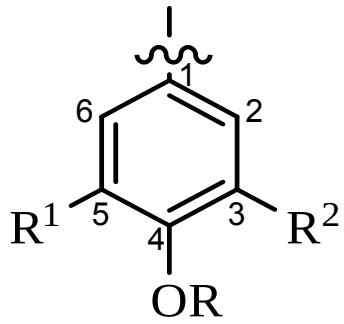
Solid State 13CNMR Spectroscopy

- **Whole cryo-milled cell wall residue**
- **Normalized for carbohydrate content**

Georgia Tech Institute of Paper Science and Technology



^{13}CNMR spectra for the control versus C3H9a and HCT30a

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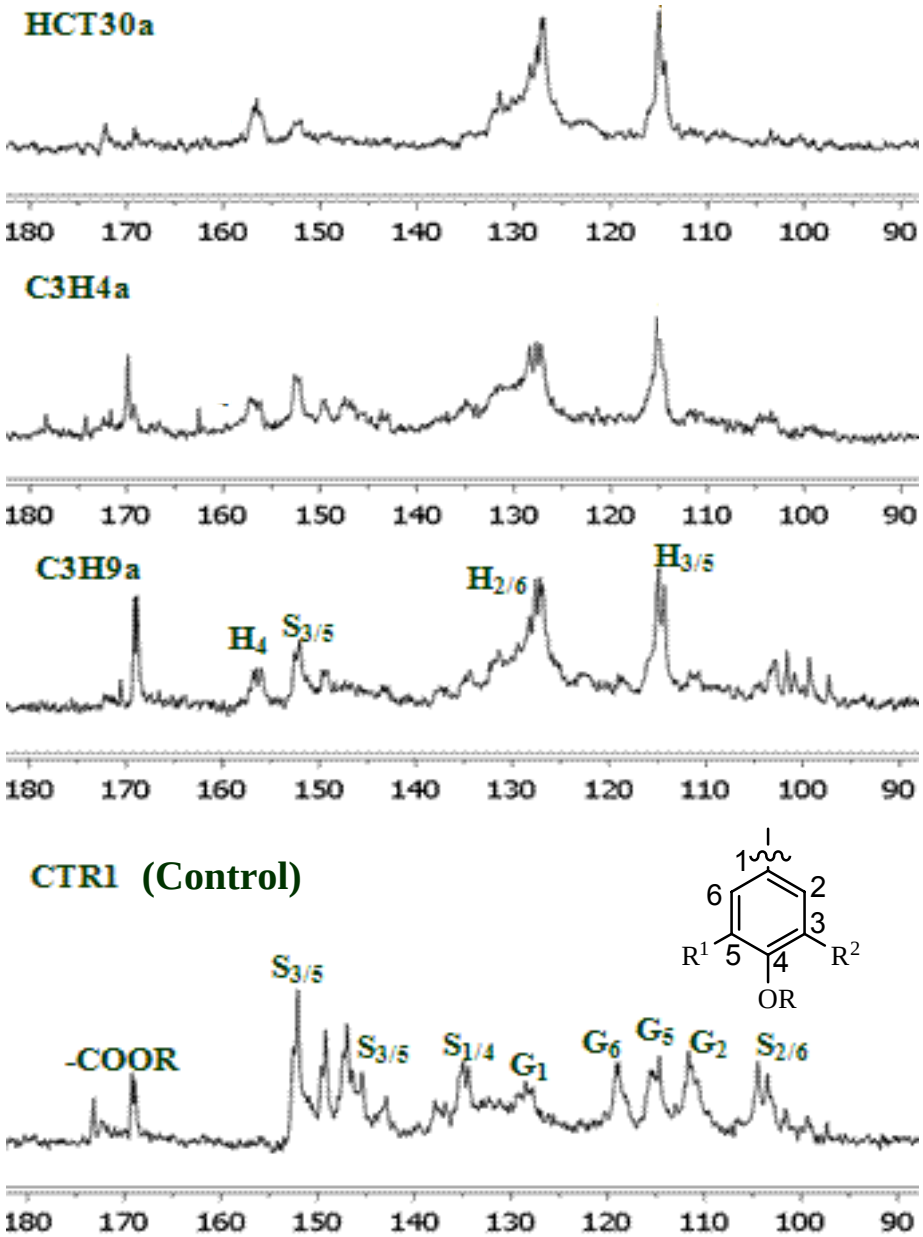
Quantitative Liquid NMR Spectroscopy

- H lignin peak easily seen in transgenic alfalfa - absent in wild type
- Less S and G lignin



Carbon NMR of ball milled, extracted lignin. HCT30a, C3H4a, C3H9a and Control (CTR1).

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Preliminary Conclusions from Detailed Analysis of Alfalfa Mutants

- Crosslinking between polymers is critical
- Altered localization does occur in mutants
- Crystallinity was not a major factor
- Multiple techniques on same samples add insights in the hands of experts

Computational Microscope – Assay and Analysis Framework